EXCRETION OF DYSENTERY BACTERIOPHAGE BY THE KIDNEYS OF MICE DURING EXPERIMENTAL DYSENTERY INFECTION

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At present, it has been proven that toxic, antigenic substances and viruses are excreted in the urine [1, 6]. L. A. Zil'ber [5] first noted the excretion of viruses by immune and unsusceptible animals, and raised the question of the role of this process in the defense of the organism from infection. In the laboratory, under his direction [8, 9], the first attempts were made to apply physiological methods for studying the mechanism of their excretion (on a model phage). A. D. Ado and co-workers, also considering the excretion of antigens as a physiological defense mechanism, expressed the opinion that the mechanism for excretion of antigens by the kidneys [2, 10, 12, 13] is similar to that for proteins [14, 15, 16].

We studied the excretion of bacteriophage by the kidneys, and the pattern of its circulation in the organism during dysentery infection in non-immune mice and mice immunized with an homologous culture or with phage.

EXPERIMENTAL METHOD

Flexner's bacteriophage (0.6 ml) was injected intravenously. After 6 hours, an intraperitoneal injection was made with dysentery culture No. 938 (500 million microbial bodies). The urine was collected in a urine-receptacle. We set up 6 clearance periods, 2, 12, 24, 48, 72, and 96 hours after injection of the phage, with a duration of 2-6 hours.

In order to intensify the diuresis, we injected 3 ml of distilled water subcutaneously. The phage level in the blood and urine was determined by titration on a solid, nutrient medium, without preliminary collection. We calculated the concentration index and the clearance index (according to the formula of van Slyke). Preliminary immunization of the mice with Flexner's dysentery culture was carried out in a three-fold manner, using doses of 250, 250 and 500 million microbial bodies subcutaneously at intervals of 7 days. Immunization with phage was also performed in three stages, injecting the culture subcutaneously every four days in doses of 0.1, 0.2 and 0.3 ml. The experiment with the immunized animals was set up 10 days after the last injection of culture of phage. In judging the validity of deviations in the mean indices, we used Pomorskii's method of fractional analysis.

In exsanguinated mice (the animals were chosen arbitrarily) we determined the seeding of the organs (liver, kidneys, spleen, lymph nodes) with phage and the pathogen. The state of reactivity of the mouse organism was judged from the reproduction of phage in the blood and organs in the case of infection, and from the concentration of antibodies (agglutinins) in the blood of the arbitrary exsanguinated, immunized animals.

EXPERIMENTAL RESULTS

In the non-immune animals (30), in the first hours after injection with culture, we noted multiplication of the phage in the blood, and an increase in its concentration (Fig. 1). The clearance index decreased by 100 times, as compared with the original figure, and rose in the course of the infection to 0.09 ml/min (by the 48th hour of observation), which is related to the change in the permeability of the kidney filtrate. The concentration index changed with the same pattern (see Table).

In the mice immunized with the dysentery bacillus (33), the original concentration of phage in the blood was lower than in the non-immune animals (see Fig. 1). In 60% of the mice there was no multiplication of the phage following the innoculation. Statistical significance of the difference (Θ) in the mean concentration of phage after

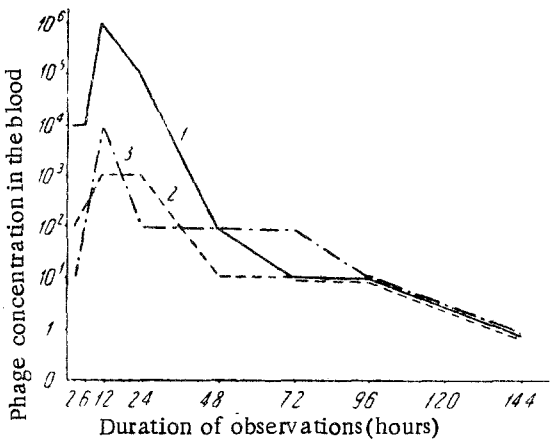

Fig. 1. Change in the concentration of dysentery bacteriophage in the blood of non-immune mice (1), and mice immunized with dysentery culture (2) and bacteriophage (3). Arbitrary scale.

the 24th hour of observation was equal to 72.96 for this group of mice. With $V_2 = 294 = 00$ and $V_1 = 1$, the indices of statistical significance are equal to 3.84 (I degree), 6.64 (II degree), and 10.84 (III degree).

Excretion of phage by the kidneys of the mice immunized with the dysentery bacillus occurred more intensely than in the non-immune animals, and the clearance index was several tens and thousands of times greater in the first days than in the last. Maximum clearance (0.9 ml/min) was noted after 48 hours (see table), and the difference from the clearance rate in the non-immune mice was completely significant: $\Theta = 10.27$.

The titer of agglutinins in the blood ranged from 1:40 to 1:2560. The intensity of excretion of phage by the kidneys did not depend on the titer of antibody; we observed marked fluctuations in the clearance index with the same antibody level.

In the mice that were immunized with bacteriophage (21), the concentration of phage in the blood was also lower than in the non-immune animals. Multiplication of phage after infection, with its active excretion in the urine, was not noted in 30% of the animals. Clearance of phage from the blood of these mice was more intense than in the non-immune subjects. The maximum clearance index after 24 hours was 0.85 ml/min, i.e. at an earlier interval than in the mice that were immunized with the culture. The difference of the means for this period is not statistically significant ($\Theta = 0.44$).

Antibodies against the microbe were observed, in weak titer, in one of the 14 mice studied. Despite the antigenic separateness of phage from the bacterial cells, we noted common patterns in the change in excretory function of the kidneys within the mice immunized with the culture and with phage. Apparently, the defense mechanisms of the organism are not limited only to specific, immunological reactions, but are connected with a change in the individual physiological functions and the system of the organism.

The diuresis in the mice of all the experimental groups remained unchanged in the first hours after inoculation (from 13 to $16 \cdot 10^{-4}$ ml/min), but by the 24th-48th hour of observation, it increased from 2 to 9 times. A comparison of the data from Figs. 1, 2, and 3 shows that the clearance index does not depend on the concentration of phage in the blood, and increases in the course of infection, as does the diuresis. However, we did not observe a direct relationship between the clearance index and the diuresis.

In order to resolve the question of specificity in the activation of the kidney excretory functioning within immunized animals, we set up experiments on 29 mice that were first immunized with typhoid culture or staphylococcal vaccine. The dynamics of phage circulation in the organism of these animals was the same as in the non-immune mice. Excretion of phage was more active. The mean clearance index ranged from 0.002 to 0.29 ml/min in the mice immunized with staphylococcal vaccine, and from 0.012 to 0.088 ml/min in those immunized with typhoid culture. The difference from the clearance indices in the non-immune mice falls in the bounds of random error.

At certain periods (up to the 48th hour of observation), the clearance indices of the mice immunized with dysentery culture substantially exceeded those of the mice immunized with the typhoid culture ($\Theta = 6.2$) or the staphylococcal vaccine ($\Theta = 4.89$). This confirms a relative specificity in the activation of excretion of phage within immune animals.

The greatest seeding of organs with phage (observations on 75 mice) was noted in the non-immune animals; the phage multiplied intensely. An almost equal degree of phage multiplication was seen in the organs of the mice immunized with phage. In a large number of the mice immunized with dysentery culture, phage multiplication was weaker or absent entirely, and it disappeared earlier, which can be explained by the more active destruction of phage and microbes in the immune organism.

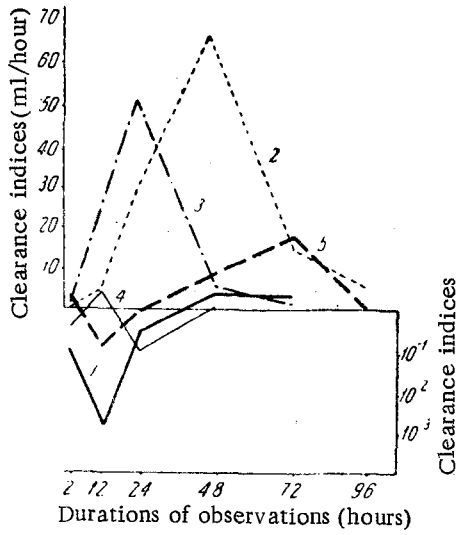

Fig. 2. Change in the mean clearance indices of bacteriophage from the blood in non-immune inoculated mice (1), and mice immunized with dysentery culture (2), bacteriophage (3), typhoid culture (4), and staphylococcal vaccine (5). Clearance indices are calculated per hour. Arbitrary scale.

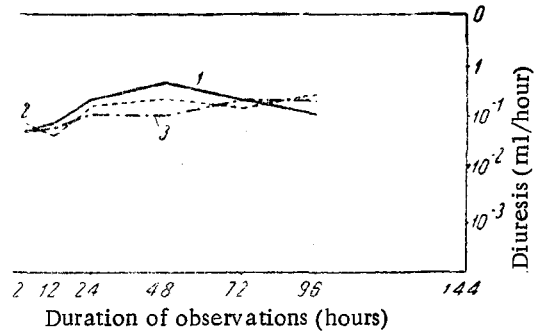

Fig. 3. Change in the mean diuresis indices for the non-immune mice (1), and the mice immunized with dysentery culture (2) and bacteriophage (3). Arbitrary scale.

In all cases, seeding of the organs with the pathogen was insignificant: of 372 sowings (without preliminary collection), pathogen was discovered in 9.

We attempted to establish a connection between the excretion of phage in the urine and the excretion of dysentery antigen, using the reaction of cold complement fixation. We investigated 2-4 mice at each period of observation in all experimental groups. The appearance of antigen in the urine coincided with the maximum clearance index (in the non-immune mice—after 48-72 hours, in the mice immunized with dysentery culture—24-48 hours, in the mice immunized with phage—after 12-24 hours). This serves as a basis for postulating that clearance of phage from the blood by the kidneys occurs in parallel with clearance of microbial antigen from the organism.

Thus, the increase in the phage clearance index and the diuresis index in the course of infection does not permit explaining excretion of phage by the kidneys solely on the basis of filtration mechanism, since it is known that an increase in the latter is caused, to a significant degree, by disruption of reabsorption in the tubules [3,4]. A number of authors use this mechanism to explain the more active excretion of antigen in immunized animals [2,10,12]. The fact that clearance is not directly dependent on the concentration of phage in the blood and on the diuresis permits postulating that participation of the renal tubules in phage excretion is not limited to the resorptive processes; apparently, phage is actively excreted into the lumen of the tubules as the organism is freed of the infection.

SUMMARY

Regularity of bacteriophage circulation in the organism, and the intensity of its excretion by the kidneys in dysentery infection, depended on the immunological body state.

In the non-immune mice, with active multiplication of phage, the clearance indices were low. In the mice immunized with the same culture or with phage, there was no multiplication; the clearance index was dozens and thousands of times greater than in the non-immune mice. There was no direct relationship between the clearance index and blood phage concentration or diuresis; there was also no connection between clearance and the blood antibody titer.

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Mean Concentration Indices and Clearance Indices in Mice

Duration of observations, (hours)	Nonimmune		Immun. with dysentery culture		Immun. with bacteriophage	
	concentration index	clearance index (ml/min)	concentration index	clearance index (ml/min)	concentration index	clearance index (ml/min)
2	0.065 ($1 \cdot 10^{-5}$-$1 \cdot 10^{-2}$)	0.002 ($2 \cdot 10^{-7}$-$3 \cdot 10^{-2}$)	1.12 ($1 \cdot 10^{-4}$-10)	0.043 ($4 \cdot 10^{-6}$-0.24)	0.6 ($1 \cdot 10^{-3}$-10)	0.02 ($2 \cdot 10^{-5}$-0.64)
12	0.0007 ($1 \cdot 10^{-7}$-$1 \cdot 10^{-2}$)	0.00003 ($5 \cdot 10^{-8}$-$2 \cdot 10^{-4}$)	2.33 ($1 \cdot 10^{-4}$-10)	0.083 ($5 \cdot 10^{-8}$-0.59)	6.23 ($1 \cdot 10^{-6}$-100)	0.23 ($2 \cdot 10^{-6}$-7.48)
24	0.082 ($1 \cdot 10^{-7}$-1.0)	0.007 ($5 \cdot 10^{-9}$-$9 \cdot 10^{-2}$)	5.76 ($1 \cdot 10^{-3}$-100)	0.46 ($8 \cdot 10^{-5}$-9.11)	13.7 ($1 \cdot 10^{-4}$-100)	0.85 ($5 \cdot 10^{-8}$-8.7)
48	0.72 ($1 \cdot 10^{-4}$-$1 \cdot 10^{-2}$)	0.09 ($4 \cdot 10^{-8}$-1.6)	10.7 ($1 \cdot 10^{-2}$-100)	0.9 ($7 \cdot 10^{-2}$-8.9)	1.8 ($1 \cdot 10^{-4}$-10)	0.09 ($7 \cdot 10^{-6}$-0.8)
72	1.16 ($1 \cdot 10^{-2}$-10)	0.08 ($5 \cdot 10^{-4}$-0.8)	2.63 ($1 \cdot 10^{-2}$-10)	0.2 ($9 \cdot 10^{-4}$-1.04)	1.0 ($1 \cdot 10^{-4}$-10)	0.08 ($7 \cdot 10^{-6}$-7.07)
96	Phage not observed		1.36 ($1 \cdot 10^{-2}$-10)	0.12 ($1 \cdot 10^{-5}$-0.74)	Phage observed in 2 mice	

The individual ranges are shown in parentheses.

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